

Practitioner's Docket No. MPI00-456P1RM

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On pages 5 and 6, please amend the paragraphs beginning on line 17 of page 5 as follows:

Figure 8 depicts a BLAST alignment of human 32544 with a consensus amino acid sequence derived from a ProDomain "KIAA0450" (PD183899) (Release 2001.1; [[http://]]www.toulouse.inra.fr/prodom.html). The lower sequence is amino acid residues 101 to 425 of the 425 amino acid consensus sequence (SEQ ID NO:10), while the upper amino acid sequence corresponds to the "KIAA0450" domain of human 32544, amino acid residues 883 to 1207 of SEQ ID NO:2.

Figures 9a-c depict a BLAST alignment of human 32544 with a consensus amino acid sequence derived from a ProDomain "phospholipase phosphodiesterase hydrolase phosphoinositide-specific 1-phosphatidylinositol-45-bisphosphate degradation tranducer lipid beta" (PD001214) (Release 2001.1; [[http://]]www.toulouse.inra.fr/prodom.html). The lower sequence is amino acid residues 2 to 159, 162 to 202, and 151 to 168 of the 219 amino acid consensus sequence (SEQ ID NOs:11-13), while the upper amino acid sequence corresponds to the "phospholipase phosphodiesterase hydrolase phosphoinositide-specific 1-phosphatidylinositol-45-bisphosphate degradation tranducer lipid beta" domain of human 32544, amino acid residues 307 to 456, 514 to 562, and 742 to 759 of SEQ ID NO:2. Figure 9a depicts the first local alignment, Figure 9b the second, and Figure 9c the third.

Figure 10 depicts a BLAST alignment of human 32544 with a consensus amino acid sequence derived from a ProDomain "phospholipase C delta calcium-binding PLC-III hydrolase phosphodiesterase lipid PLC-delta-1 1-phosphatidylinositol-45-bisphosphate" (PD186804) (Release 2001.1; [[http://]]www.toulouse.inra.fr/prodom.html). The lower sequence is amino acid residues 14 to 194 of the 203 amino acid consensus sequence (SEQ ID NO:14), while the upper amino acid sequence corresponds to the "phospholipase C delta calcium-binding PLC-III hydrolase phosphodiesterase lipid PLC-delta-1 1-phosphatidylinositol-45-bisphosphate" domain of human 32544, amino acid residues 41 to 214 of SEQ ID NO:2.

Figure 11 depicts a BLAST alignment of human 32544 with a consensus amino acid sequence derived from a ProDomain "phospholipase binding C KDA-INS145P3 K10F12.3" (PD023751) (Release 2001.1; [[http://]]www.toulouse.inra.fr/prodom.html). The lower sequence is amino acid residues 2 to 135 of the 136 amino acid consensus sequence (SEQ ID NO:15),

Practitioner's Docket No. MPI00-456P1RM

USSN: 09/927,112

while the upper amino acid sequence corresponds to the "phospholipase binding C KDA-INS145P3 K10F12.3" domain of human 32544, amino acid residues 174 to 304 of SEQ ID NO:2.

Figure 12 depicts a BLAST alignment of human 32544 with a consensus amino acid sequence derived from a ProDomain "FLJ12548 similar FIS cDNA phosphatidylinositol-45-bisphosphate NT2RM4000657 delta phosphodiesterase weakly 1-" (PD308221) (Release 2001.1; [[http://]]www.toulouse.inra.fr/prodom.html). The lower sequence is amino acid residues 2 to 93 of the 187 amino acid consensus sequence (SEQ ID NO:16), while the upper amino acid sequence corresponds to the "FLJ12548 similar FIS cDNA phosphatidylinositol-45-bisphosphate NT2RM4000657 delta phosphodiesterase weakly 1-" domain of human 32544, amino acid residues 851 to 944 of SEQ ID NO:2.

On page 7, please amend the paragraph beginning on line 28 as follows:

For general information regarding PFAM identifiers, PS prefix and PF prefix domain identification numbers, refer to Sonnhammer et al. (1997) *Protein* 28:405-420 and <http://www.psc.edu/general/software/packages/pfam/pfam.html> www.psc.edu/general/software/packages/pfam/pfam.html

On page 8, please delete the paragraph beginning on line 1 as follows:

~~A plasmid containing the nucleotide sequence encoding human 32544 was deposited with American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, on _____ and assigned Accession Number _____. This deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This deposit was made merely as a convenience for those of skill in the art and is not an admission that a deposit is required under 35 U.S.C. §112.~~

Practitioner's Docket No. MPI00-456P1RM

USSN: 09/927,112

On page 8, please amend the paragraph beginning on line 8 as follows:

To identify the presence of a "phospholipase family" domain in a 32544 protein sequence (Pfam accession number 00387 and 00388), and make the determination that a polypeptide or protein of interest has a particular profile, the amino acid sequence of the protein can be searched against a database of HMMs (e.g., the Pfam database, release 2.1) using the default parameters ([[http://]]www.sanger.ac.uk/Software/Pfam/HMM_search). For example, the hmmsf program, which is available as part of the HMMER package of search programs, is a family specific default program for MILPAT0063 and a score of 15 is the default threshold score for determining a hit. Alternatively, the threshold score for determining a hit can be lowered (e.g., to 8 bits). A description of the Pfam database can be found in Sonhammer et al., (1997) *Proteins* 28(3):405-420 and a detailed description of HMMs can be found, for example, in Gribskov et al., (1990) *Meth. Enzymol.* 183:146-159; Gribskov et al., (1987) *Proc. Natl. Acad. Sci. USA* 84:4355-4358; Krogh et al., (1994) *J. Mol. Biol.* 235:1501-1531; and Stultz et al., (1993) *Protein Sci.* 2:305-314, the contents of which are incorporated herein by reference.

On pages 12 and 13, please amend the paragraph beginning on line 22 of page 12 as follows:

To identify the presence of a "PH" domain, "PLC-X domain," "PLC-Y domain," or a "C2" domain in a 32544 protein sequence, and make the determination that a polypeptide or protein of interest has a particular profile, the amino acid sequence of the protein can be searched against a database of HMMs (e.g., the Pfam database, release 2.1) using the default parameters ([[http://]]www.sanger.ac.uk/Software/Pfam/HMM_search). For example, the hmmsf program, which is available as part of the HMMER package of search programs, is a family specific default program for MILPAT0063 and a score of 15 is the default threshold score for determining a hit. Alternatively, the threshold score for determining a hit can be lowered (e.g., to 8 bits). A description of the Pfam database can be found in Sonhammer et al. (1997) *Proteins* 28(3):405-420 and a detailed description of HMMs can be found, for example, in Gribskov et al. (1990) *Meth. Enzymol.* 183:146-159; Gribskov et al. (1987) *Proc. Natl. Acad. Sci. USA* 84:4355-4358; Krogh et al. (1994) *J. Mol. Biol.* 235:1501-1531; and Stultz et al. (1993) *Protein Sci.* 2:305-314,

Practitioner's Docket No. MPI00-456P1RM

USSN: 09/927,112

the contents of which are incorporated herein by reference. A search was performed against the HMM database resulting in the identification of a "PH domain" domain in the amino acid sequence of human 32544 at about residues 44-151 of SEQ ID NO:2 (see Figure 3); a "C2 domain" in the amino acid sequence of human 32544 at about residues 756-848 of SEQ ID NO:2 (see Figure 7); an EF hand domain in the amino acid sequence of human 32544 at about residues 169 to 197 of SEQ ID NO:2 (see Fig. 4); a "PLC-Y domain" in the amino acid sequence of human 32544 at about residues 621-736 of SEQ ID NO:2 (see Figure 6); and a "PLC-X domain" in the amino acid sequence of human 32544 at about residues 323-468 of SEQ ID NO:2 (see Figure 5).

On pages 13 and 14, please amend the paragraph beginning on line 23 of page 13 as follows:

The phospholipase domain is homologous to ProDom family PD183899 ("KIAA0450" SEQ ID NO:10, ProDomain Release 2001.1; www.toulouse.inra.fr/prodom.html). <http://www.toulouse.inra.fr/prodom.html>. An alignment of the phospholipase domain (amino acids 883 to 1207 of SEQ ID NO:2) of human 32544 with a consensus amino acid sequence (SEQ ID NO:10) derived from a hidden Markov model is depicted in Figure 8. The consensus sequence for SEQ ID NO:8 is 77% identical over amino acids 883 to 1207 of SEQ ID NO:2 as shown in Figure 8.

The phospholipase domain is homologous to ProDom family PD001214 ("phospholipase phosphodiesterase hydrolase phosphoinositide-specific 1-phosphatidylinositol-45-bisphosphate degradation tranducer lipid beta" SEQ ID NOs:11-13, ProDomain Release 2001.1; www.toulouse.inra.fr/prodom.html) <http://www.toulouse.inra.fr/prodom.html>. An alignment of the phospholipase domain (amino acids 307 to 456, 514 to 562, and 742 to 759 of SEQ ID NO:2) of human 32544 with a consensus amino acid sequence (SEQ ID NO:10) derived from a hidden Markov model is depicted in Figure 9. The consensus sequence for SEQ ID NO:11 is 55% identical over amino acids 307 to 456 of SEQ ID NO:2; the consensus sequence for SEQ ID NO:12 is 30% identical over amino acids 514 to 562 of SEQ ID NO:2; and the consensus

Practitioner's Docket No. MPI00-456P1RM

USSN: 09/927,112

sequence for SEQ ID NO:13 is 33% identical over amino acids 742 to 759 of SEQ ID NO:2 as shown in Figure 9.

The phospholipase domain is homologous to ProDom family PD186804 ("phospholipase C delta calcium-binding PLC-III hydrolase phosphodiesterase lipid PLC-delta-1 1-phosphatidylinositol-45-bisphosphate" SEQ ID NO:14, ProDomain Release 2001.1; www.toulouse.inra.fr/prodom.html <http://www.toulouse.inra.fr/prodom.html>). An alignment of the phospholipase domain (amino acids 41 to 214 of SEQ ID NO:2) of human 32544 with a consensus amino acid sequence (SEQ ID NO:14) derived from a hidden Markov model is depicted in Figure 10. The consensus sequence for SEQ ID NO:14 is 37% identical over amino acids 41 to 214 of SEQ ID NO:2 as shown in Figure 10.

The phospholipase domain is homologous to ProDom family PD023751 ("phospholipase binding C KDA-INS145P3 K10F12.3" SEQ ID NO:15, ProDomain Release 2001.1; www.toulouse.inra.fr/prodom.html <http://www.toulouse.inra.fr/prodom.html>). An alignment of the phospholipase domain (amino acids 174 to 304 of SEQ ID NO:2) of human 32544 with a consensus amino acid sequence (SEQ ID NO:15) derived from a hidden Markov model is depicted in Figure 11. The consensus sequence for SEQ ID NO:14 is 39% identical over amino acids 174 to 304 of SEQ ID NO:2 as shown in Figure 11.

The phospholipase domain is homologous to ProDom family PD308221 ("FLJ12548 similar FIS cDNA phosphatidylinositol-45-bisphosphate NT2RM4000657 delta phosphodiesterase weakly 1-" SEQ ID NO:16, ProDomain Release 2001.1; www.toulouse.inra.fr/prodom.html <http://www.toulouse.inra.fr/prodom.html>). An alignment of the phospholipase domain (amino acids 851 to 944 of SEQ ID NO:2) of human 32544 with a consensus amino acid sequence (SEQ ID NO:15) derived from a hidden Markov model is depicted in Figure 12. The consensus sequence for SEQ ID NO:16 is 39% identical over amino acids 851 to 944 of SEQ ID NO:2 as shown in Figure 12.

On page 30, please amend the paragraph beginning on line 1 as follows:

A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of 32544 (e.g., the sequence of SEQ ID NO:1[[,]] or SEQ ID NO:3, or the nucleotide

Practitioner's Docket No. MPI00-456P1RM

USSN: 09/927,112

~~sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____~~
without abolishing or more preferably, without substantially altering a biological activity, whereas an "essential" amino acid residue results in such a change. For example, amino acid residues that are conserved among the polypeptides of the present invention, e.g., those present in the phospholipase family domain, are predicted to be particularly unamenable to alteration.

On page 30, please amend the paragraph beginning on line 9 as follows:

A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in a 32544 protein is preferably replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a 32544 coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for 32544 biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NO:1[[,]] ~~or SEQ ID NO:3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____~~, the encoded protein can be expressed recombinantly and the activity of the protein can be determined.

On page 32, please amend the paragraph beginning on line 3 as follows:

The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and

Practitioner's Docket No. MPI00-456P1RM

USSN: 09/927,112

Wunsch (*J. Mol. Biol.* (48):444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at [[http://]]www.gcg.com), using either a Blosum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available at [[http://]]www.gcg.com), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. A particularly preferred set of parameters (and the one that should be used if the practitioner is uncertain about what parameters should be applied to determine if a molecule is within a sequence identity or homology limitation of the invention) is using a Blosum 62 scoring matrix with a gap open penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5.

On pages 32 and 33, please amend the paragraph beginning on line 21 of page 32 as follows:

The nucleic acid and protein sequences described herein can be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, et al., (1990) *J. Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to 32544 nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to 32544 protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See [[http://]]www.ncbi.nlm.nih.gov.

Practitioner's Docket No. MPI00-456P1RM

USSN: 09/927,112

On page 34, please amend the paragraph beginning on line 3 as follows:

In one embodiment, an isolated nucleic acid molecule of the invention includes the nucleotide sequence shown in SEQ ID NO:1, ~~or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____~~, or a portion of any of these nucleotide sequences. In one embodiment, the nucleic acid molecule includes sequences encoding the human 32544 protein (i.e., "the coding region", from nucleotides 435-4055 of SEQ ID NO:1, not including the terminal codon), as well as 5' untranslated sequences (nucleotides 1-434 of SEQ ID NO:1). Alternatively, the nucleic acid molecule can include only the coding region of SEQ ID NO:1 (e.g., nucleotides 435-4055 of SEQ ID NO:1, corresponding to SEQ ID NO:3) and, e.g., no flanking sequences which normally accompany the subject sequence. In another embodiment, the nucleic acid molecule encodes a sequence corresponding to the mature protein of SEQ ID NO:2.

On page 34, please amend the paragraph beginning on line 14 as follows:

In another embodiment, an isolated nucleic acid molecule of the invention includes a nucleic acid molecule which is a complement of the nucleotide sequence shown in SEQ ID NO:1[.] ~~or SEQ ID NO:3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____~~, or a portion of any of these nucleotide sequences. In other embodiments, the nucleic acid molecule of the invention is sufficiently complementary to the nucleotide sequence shown in SEQ ID NO:1[.] ~~or SEQ ID NO:3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____~~ such that it can hybridize to the nucleotide sequence shown in SEQ ID NO:1[.] ~~or SEQ ID NO:3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____~~, thereby forming a stable duplex.

On pages 34 and 35, please amend the paragraph beginning on line 24 of page 34 as follows:

In one embodiment, an isolated nucleic acid molecule of the present invention includes a nucleotide sequence which is at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%,

Practitioner's Docket No. MPI00-456P1RM

USSN: 09/927,112

93%, 94%, 95%, 96%, 97%, 98%, 99%, or more homologous to the nucleotide sequence shown in SEQ ID NO:1[[,]] or SEQ ID NO:3, ~~or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____~~. In the case of an isolated nucleic acid molecule which is longer than or equivalent in length to the reference sequence, e.g., SEQ ID NO:1, or SEQ ID NO:3, the comparison is made with the full length of the reference sequence. Where the isolated nucleic acid molecule is shorter than the reference sequence, e.g., shorter than SEQ ID NO:1, or SEQ ID NO:3, the comparison is made to a segment of the reference sequence of the same length (excluding any loop required by the homology calculation).

On page 35, please amend the paragraph beginning on line 6 as follows:

A nucleic acid molecule of the invention can include only a portion of the nucleic acid sequence of SEQ ID NO:1[[,]] or SEQ ID NO:3, ~~or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____~~. For example, such a nucleic acid molecule can include a fragment which can be used as a probe or primer or a fragment encoding a portion of a 32544 protein, e.g., an immunogenic or biologically active portion of a 32544 protein. A fragment can comprise e.g., nucleotides 275 to 598 of SEQ ID NO:1, which encodes a PH domain of human 32544; nucleotides 1112 to 1549 of SEQ ID NO:1, which encodes the PLC-X domain of human 32544; or nucleotides 2006 to 2353 of SEQ ID NO:1, which encodes the PLC-Y domain of human 32544. The nucleotide sequence determined from the cloning of the 32544 gene allows for the generation of probes and primers designed for use in identifying and/or cloning other 32544 family members, or fragments thereof, as well as 32544 homologues, or fragments thereof, from other species.

On page 36, please amend the paragraph beginning on line 1 as follows:

32544 probes and primers are provided. Typically a probe/primer is an isolated or purified oligonucleotide. The oligonucleotide typically includes a region of nucleotide sequence that hybridizes under stringent conditions to at least about 7, 12 or 15, preferably about 20 or 25, more preferably about 30, 35, 40, 45, 50, 55, 60, 65, or 75 consecutive nucleotides of a sense or

Practitioner's Docket No. MPI00-456P1RM

USSN: 09/927,112

antisense sequence of SEQ ID NO:1[.] or SEQ ID NO:3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number ____, or of a naturally occurring allelic variant or mutant of SEQ ID NO:1[.] or SEQ ID NO:3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number ____.

On page 37, please amend the paragraph beginning on line 8 as follows:

A nucleic acid fragment encoding a "biologically active portion of a 32544 polypeptide" can be prepared by isolating a portion of the nucleotide sequence of SEQ ID NO:1[.] or SEQ ID NO:3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number ____, which encodes a polypeptide having a 32544 biological activity (e.g., the biological activities of the 32544 proteins as described herein), expressing the encoded portion of the 32544 protein (e.g., by recombinant expression *in vitro*) and assessing the activity of the encoded portion of the 32544 protein. For example, a nucleic acid fragment encoding a biologically active portion of 32544 includes a phospholipase family domain (e.g., about nucleotides 1401-1838 of SEQ ID NO:1). A nucleic acid fragment encoding a biologically active portion of a 32544 polypeptide, may comprise a nucleotide sequence which is greater than 300-1200 or more nucleotides in length.

On page 37, please amend the paragraph beginning on line 20 as follows:

In preferred embodiments, nucleic acids include a nucleotide sequence which is about 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200 nucleotides in length and hybridizes under stringent hybridization conditions to a nucleic acid molecule of SEQ ID NO:1[.] or SEQ ID NO:3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number ____.

Practitioner's Docket No. MPI00-456P1RM

USSN: 09/927,112

On pages 37 and 38, please amend the paragraph beginning on line 27 of page 37 as follows:

The invention further encompasses nucleic acid molecules that differ from the nucleotide sequence shown in SEQ ID NO:1[,,] or SEQ ID NO:3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____. Such differences can be due to degeneracy of the genetic code (and result in a nucleic acid which encodes the same 32544 proteins as those encoded by the nucleotide sequence disclosed herein. In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a protein having an amino acid sequence which differs, by at least 1, but less than 5, 10, 20, 50, or 100 amino acid residues that shown in SEQ ID NO:2. If alignment is needed for this comparison the sequences should be aligned for maximum homology. "Looped" out sequences from deletions or insertions, or mismatches, are considered differences.

On page 38, please amend the paragraph beginning on line 18 as follows:

In a preferred embodiment, the nucleic acid differs from that of SEQ ID NO:1[,,] or SEQ ID NO:3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, e.g., as follows: by at least one but less than 10, 20, 30, or 40 nucleotides; at least one but less than 1%, 5%, 10% or 20% of the in the subject nucleic acid. If necessary for this analysis the sequences should be aligned for maximum homology. "Looped" out sequences from deletions or insertions, or mismatches, are considered differences.

On page 39, please amend the paragraph beginning on line 16 as follows:

Moreover, nucleic acid molecules encoding other 32544 family members and, thus, which have a nucleotide sequence which differs from the 32544 sequences of SEQ ID NO:1[,,] or SEQ ID NO:3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number ____ are intended to be within the scope of the invention.

Practitioner's Docket No. MPI00-456P1RM

USSN: 09/927,112

On pages 71 and 72, please amend the paragraph beginning on line 27 of page 71 as follows:

The isolated mRNA can be used in hybridization or amplification assays that include, but are not limited to, Southern or Northern analyses, polymerase chain reaction analyses and probe arrays. One preferred diagnostic method for the detection of mRNA levels involves contacting the isolated mRNA with a nucleic acid molecule (probe) that can hybridize to the mRNA encoded by the gene being detected. The nucleic acid probe can be, for example, a full-length 32544 nucleic acid, such as the nucleic acid of SEQ ID NO:1, ~~or the DNA insert of the plasmid deposited with ATCC as Accession Number _____, or a portion thereof, such as an oligonucleotide of at least 7, 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to 32544 mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays are described herein.~~